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Entomopathogens: Theory and practice

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1. Entomopathogens: Knowledge and potential

Entomopathogens are the pathogens of insects, in other words those microorganisms or microscopic infectious agents like viruses, capable of causing them a disease possibly leading to their death. Beyond this simple and obvious definition, entomopathogens can be seen as those microbes that evolved with their hosts developing the ability to overcome their immune barriers, successfully exploiting them as useful nutritional resources. While we might agree that a primary ecosystem role of microbials (especially soil microorganisms like bacteria and fungi) is as decomposers of organic matter, we might infer that speciation has led to the establishment of species that became capable of interacting to their own advantage with living organisms for which they became pathogens. Within the multifaceted network of biocenotic relationships in the ecosystem, we can imagine the complexity of interactions between the invisible microbial world and the relatively larger insect world (Douglas, 2009).

Only careful observation, supported by the aid of technologies and scientific equipment that have over time become increasingly sophisticated and advanced, has made it possible to study and interpret the mechanisms through which a pathogen interacts with its host, giving a clear glimpse of possible practical applications, especially in the field of pest management. In fact, although in a natural ecosystem we assume the existence of well-established balances between entomopathogens and insect populations, conditions of disequilibrium in favour of the latter typically characterise man-made agro-ecosystems. Accordingly, pest outbreaks in such environments are frequent and, particularly in the population density peak phases, are associated with epidemic phenomena caused by entomopathogenic agents that find ideal conditions to spread and thus act containing pest populations. Beyond these special cases, entomopathogens are always present in the environment contributing to the so-called "environmental resistance" regulating the biotic potential of pest species. Although we can identify these common characteristics, among entomopathogens we find very different living forms such as viruses, bacteria, fungi, microsporidia, and a particular category of nematodes (Tanada and Kaya, 2012).

This fascinating world of entomopathogens is also represented by a wide inter- and intraspecific variability that represents considerable opportunities for practical applications. Although numerous studies have been conducted in recent decades by a dedicated scientific community, many aspects still need to be clarified regarding the mechanisms of action and pathogenic processes involving a plethora of proteins and other insecticidal compounds, enzymes and virulence factors. Added to this is the continual discovery of new strains of entomopathogens with previously unknown gene traits that give them special biological properties that can be leveraged for the development of new biosolutions. Hence the industrial interest that has always supported research and development in this area by foreseeing the changing needs of pest management and introducing ever newer and more sophisticated microbial-based products to the market.

2. Entomopathogenic viruses

Among viruses that establish relationships with insects, of particular interest is the *Baculoviridae* family, especially in relation to its potential for biological control applications. A baculovirus consists of a circular double-stranded DNA genome enclosed in a capsid and an outer lipid envelope. During infection cycle of baculoviruses, two virion phenotypes are produced, (i) the so-called occlusion-derived virus (ODV), which are typically contained within crystalline occlusion bodies (OBs) and initiate midgut infection after peroral ingestion of the OB, and (ii) the budded virus (BV), which spread the infection from cell to cell in an infected larvae. The OB morphology allowed the distinction between nucleopolyhedroviruses or NPVs (polyhedron-shape), associated with Lepidoptera, Hymenoptera and Diptera, and granuloviruses or GVs (granular-shape) of Lepidoptera. Genome sequencing and analyses have accelerated knowledge about insect viruses, increasing understanding of their biology and the mechanisms of co-evolution with their hosts

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(Herniou and Jehle, 2007). These studies revealed the presence of 38 conserved core genes related to baculovirus essential biology and other genes providing the virus with selective advantages in the interaction with the environment or in the virulence against the host. Studies on genomics and gene expression are disclosing a variety of factors acting at progressive levels of the infection that normally initiates by ingestion of contaminated matrices and involves envelope proteins acting as *per os* infectivity factors. The spread of the OBs in the environment after causing the death and liquefaction of the host is often ensured by mechanisms of interference with host behaviour (Clem and Passarelli, 2013).

Studies in these areas are producing new insights that not only provide basic knowledge for understanding the ecological role of insect viruses, but also generate valuable information for exploitation from an industrial perspective (Haase et al., 2015). Accordingly, numerous baculovirus-based plant protection products are now commercially available, which aligns with their recognised good ecotoxicological profile in the authorisation regulatory system. Studies on virus diversity (Sutela et al., 2024) and their compatibility with other biocontrol agents (Koller et al., 2024) are included in the present Special Issue.

3. Entomopathogenic bacteria

Bacteria are unicellular Prokaryotae that have evolved a wide variety of interactions with insects, including mutualistic symbiosis assisting food digestion processes, or intimate relationships in which the insect becomes a vector for plant or animal pathogens. Although several bacteria reside in the body of insects, especially in the intestine, only a few have evolved as pathogens. While the generality of bacteria can easily enter the intestinal tract through the ingestion of various organic matrices (e.g. leaves) on which the bacteria or their propagules are spread, only pathogens can cross the intestinal barrier, using weapons that the evolutionary process has provided them with. The host haemocoel represents the site to be reached, where the bacterium can find nourishment and resources for its proliferation in the hemolymph, which normally leads to septicaemia and insect death. Among the wide variety of species, we distinguish obligate pathogens that are only able to carry out their pathogenic cycle within the host, facultative pathogens that are also able to develop in the environment outside the host, and opportunistic bacteria that only behave as pathogens when conditions are particularly favourable. This aligns with the wide variety of microbial species and strains and the bioactive molecules they produce (Ruiu, 2015).

Bacterial genetic information is found in double-stranded DNA molecules (chromatin free) and in plasmids, whereby mutations and acquisition of genetic material (by transformation, transduction and conjugation) ensure the genetic variations required for adaptation to the changing environment.

Of relevance, especially for the pest management application potential is the Bacillaceae family, including the well-known species *Bacillus thuringiensis*, *Lysinibacillus sphaericus.*, *Brevibacillus laterosporus* and *Paenibacillus* spp. In these spore-formers, the production of parasporal bodies (crystals) during sporulation is common. Crystals contain insecticidal proteins (Cry) normally active against a specific target range. Other bacterial proteins and non-protein compounds are instead toxic to a broader pest spectrum. Due to a significant diversity of bacterial pesticidal proteins, further fuelled by the discovery of new coding genes and gene sequences harboured by new isolates, different classification systems have been developed, lately based on protein structure (Crickmore et al., 2021).

Alongside these bacterial species that have found greater industrial success, there are several Gram-negatives on which the level of attention has recently increased in view of novel application prospects. These include several Gammaproteobacteria species in the genera *Serratia* and *Pseudomonas* (Jurat-Fuentes and Jackson, 2012) and the insecticidal compounds and virulence factors they produce. Consistently, this Special Issue, highlights the potential of chitinases, specifically produced by an insect-isolated strain of *Serratia ficaria* (Bakirdogen and Eroglu, 2024). Successfully used on an industrial level, interest is also growing in Betaproteobacteria like the more recently discovered *Burkholderia* and *Chromobacterium* species (Ruiu, 2015).

4. Entomopathogenic fungi

The use entomopathogenic fungi for the biological control of arthropod pests of agricultural, forestry, medical-veterinary, and even urban interest has made considerable progress during the XXI century, as evidenced by the number of commercial products available and under development (Quesada-Moraga et al., 2020). This growing interest in entomopathogenic fungi relates to their natural presence in the soil, and in the arthropod populations they regulate establishing unique relationships with plants (Quesada-Moraga, 2020; Quesada-Moraga et al., 2020). The species of entomopathogenic fungi, which are highly diverse, are distributed across many classes of Mycota, whereas the two most important groups are the Entomophthoromycota, whose members cause natural epizootics, but their practical application is currently limited due to their obligate biotrophy and therefore their difficulty of being produced in artificial media, and the Hypocrealean fungi (Ascomycota), which contain the largest number of entomopathogenic species, being suitable for development and use in inoculative and inundative microbial pest control, with key genus such as *Beauveria*, *Metarhizium*, *Isaria* and *Akanthomyces* being commercially available (Quesada-Moraga et al., 2020). Entomopathogenic ascomycetes are unique among entomopathogenic microorganisms in their mode of action by contact, through the arthropod integument. Conidia adhere to the cuticle, germinate, and form specific penetration structures enabling the fungus to penetrate through the integument and, after overcoming the host insect's cellular and humoral defensive responses, to cause the death of the insect host mainly by invading its tissues and organs, whereas different less frequent modes of action have been described in the last years. The insect's death leads to the saprophytic growth of the fungus (Quesada-Moraga et al., 2020). Under favourable conditions, the fungus grows out from the cadaver, produces conidiophores and conidia, leading to the dissemination of fungal propagules to new arthropod hosts (Quesada-Moraga et al., 2020). By penetrating through the integument, entomopathogenic fungi become a key microbial control tool of insects with piercing-sucking mouthparts, soil dwelling insect pests, insects with chewing mouthparts for which viral or bacterial diseases are unknown or ineffective, locusts and grasshoppers, synanthropic insects such as termites and cockroaches and all invasive insect pests among others (Quesada-Moraga et al., 2020). Entomopathogenic ascomycetes have been reported to be compatible with other microbials and with predators and parasitoids in IPM programs and their multifunctional lifestyles as rhizosphere competent and endophytic microorganisms has altered the rationale behind their use as biocontrol agents in agriculture providing new approaches to crop protection and crop production (Quesada-Moraga et al., 2022, 2023). In this *Crop Protection* Special Issue there are key contributions to the use of entomopathogenic fungi for invasive pest control, including the whitefly *Bemisia tabaci* (Li et al., 2024) and the red palm weevil *Rhynchophorus ferrugineus* (Sabbahi and Hock, 2023), to the possible impact of the standard pesticide application machinery on their viability (Beltrán-Martí et al., 2024) and to the new pest control strategies related to their endophytic behaviour (Darsouei et al., 2024). Presently, there is a worldwide research need to unravel the key issues on entomopathogenic ascomycete mass production and formulation, compatibility with other pest control means, environmental competence, and pest control impact to become an increasingly important alternative to chemical pesticides. In addition, future research should focus on evaluating the economic value of the ecological services provided by entomopathogenic fungi and on better exploring their multifunctional lifestyles in sustainable agriculture (Quesada-Moraga et al., 2022, 2023).

5. Entomopathogenic nematodes

Most nematodes are free-living organisms found in soil or in water. One quarter of all nematodes are parasites of plants or animals (Blaxter and Koutsovoulos, 2015), and, among the latter, some species are associated in various ways with insects. These relationships range from phoresy to symbiosis, and from commensalism to facultative or obligate parasitism (Blaxter et al., 1998; Schmid-Hempel, 2008). Among more than 30 families of nematodes associated with insects, the families Steinernematidae Filipjev, 1934, and Heterorhabditidae Poinar, 1976 (Order Rhabditida), are the ones that arouse the most interest and from a practical point of view in terms of hexapod control, nematodes within these two families are more accurately called entomopathogens because they exert their action in association with symbiotic bacteria (Kaya and Gaugler, 1993). Entomopathogenic nematodes (EPNs) are ubiquitous soil organisms and live in obligate association with bacteria in the soil killing a wide range of insects, among which soil-dwelling pests (Tarasco et al., 2015). In the past three decades, many surveys were conducted in numerous countries and new heterorhabditid and steinernematid species (more than one hundred) have been identified (Tarasco et al., 2017, 2023).

For the biological cycle, it is the third stage, also known as the Infective Juvenile (IJ) stage, that initiates the infection. Having identified the target host, IJs penetrate its body, preferably through natural openings (i.e., the mouth, anus, or stigmas), after drilling its tracheae or intestines (Ishibashi and Kondo, 1990). Once in the hemolymph, the IJs release the symbiotic bacteria present in their gut: the *Steinernema* release *Xenorhabdus* spp. and the *Heterorhabditis* release *Photorhabdus* spp. The nematodes act like a small syringe to inject the bacteria. In the hemolymph, these bacteria multiply rapidly and produce a wide range of toxins and exoenzymes that kill the host, turning its tissues into a kind of soup on which the nematodes feed to reach the adult stage after four stages of development. Antimicrobial substances that are also secreted promote the development of symbiotic bacteria and nematodes (Ji et al., 2004). Additionally, the nematodes themselves make significant contributions to killing the host (Chang et al., 2019). When they reach the adult stage, *Steinernema* spp. mate and produce successive generations, whereas IJs of the *Heterorhabditis* spp. develop into self-fertilising hermaphroditic females that will produce males and females in the next generation. The cycle is completed within a few days, and hundreds of thousands of new IJs will emerge from the now-destroyed host in search of new victims. *Steinernema* and *Heterorhabditis* spp. parasitize a wide range of insect species, and usually the death of the host occurs quickly, as 48 h is sufficient time for the bacteria to take effect. Recently, in the context of this nematode–bacteria symbiosis, an infectious contribution has also been demonstrated for other bacteria (Ogier et al., 2020), such as the bacterium *Pseudomonas protegens* (Ruiu et al., 2022). EPNs are considered effective biological control agents and serve as an alternative to the chemical control of insect pests. Commercial products containing EPNs, (especially *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema feltiae*, and *S. carpocapsae* species) have been and are successfully used to control various species of insects (mainly Heteroptera, Lepidoptera, Coleoptera, Diptera, and Hymenoptera) (De Luca et al., 2015), showing that biological control with EPNs is a real alternative and offers many advantages over chemicals, such as end user safety, minimal damage to natural enemies, and lack of environmental pollution, which are essential conditions for an advanced IPM strategy (Tarasco et al., 2023).

The successful use of EPNs in the microbial control of pests implies their efficient industrial production process and delivery to the target. Consistently, in this Special Issue the results of experiments studying the optimization of EPN mass culture (Kumari et al., 2024; Ulu and Susurluk, 2024) and their robot-assisted precision application (Erdoğan et al., 2023) are presented. The ongoing search for new EPN strains with potential against specific target pests is also highlighted (Rakubu et al., 2024).

6. Conclusions and perspectives

The present Special Issue dedicated to entomopathogens and biological control brought together contributions from experts about pathogenic bacteria, fungi, viruses, and nematodes. Research outputs from this initiative outlined the pivotal importance of continuous screening of novel entomopathogenic organisms, which is leading to the discovery of new microbial strains and genes implied in pathogenesis. Further research is still needed to shed light on the complex mode of action characterising entomopathogens attacking pests and vectors of agricultural importance. We highlight the urgent need of future studies on volatile substances (VOCs) emitted by microbial agents, such as entomopathogenic fungi and their effects on arthropods, studies on exudates that may exert an attraction on the entomopathogenic nematodes, as well as studies on the antibiotic properties of metabolites produced by their symbiotic bacteria. In addition to their profile compatible with organic farming, entomopathogens represent an excellent tool to be used in Integrated Pest Management programmes and with a view to resistance management. Of note, the legislative framework in different countries worldwide is evolving to promote the employment of biosolutions including entomopathogens, which foster basic and applied studies in this field. Overall, we sincerely thank all the contributors to the *Crop Protection* Special Issue "*Entomopathogens*", and we hope that the present volume will inspire further research to face the above-mentioned challenges.

Declaration of competing interest

The authors declare no competing interests. Giovanni Benelli is the Special Content Editor of *Crop Protection* and this article was independently handled by another Editor.

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